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(71) Applicant: Hitachi, Ltd.
5-1, Marunouchi 1-chome
Chiyoda-ku Tokyo 100(JP)

(72) Inventor: Furatu, Yoshiteru
663 Ichige
Katsuta-shi(JP)

(72) Inventor: Nomura, Yasushi
991-15 Motoyoshidacho
Mito-shi(JP)

(74) Representative: Paget, Hugh Charles Edward et al,
MEWBURN ELLIS & CO. 70/72 Chancery Lane
London WC2A 1AD(GB)

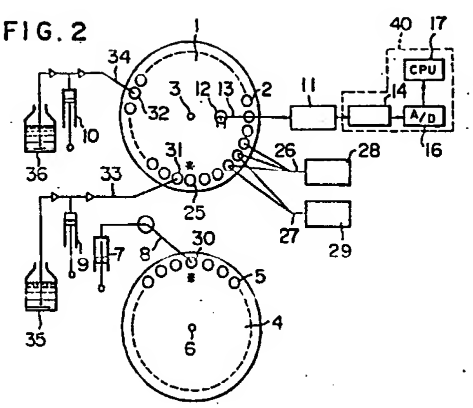
(54) **Method for optically analyzing a plurality of analysis items.**

(57) Characteristics of two items in a sample are determined by sequentially adding first and second reagents and optically measuring characteristics of the first and second reaction solutions so obtained.

For example, transparent containers (2) are supported on a rotatable disc (1). A fixed volume of serum is introduced into one of the containers (2) by means of a pipette (9). The first reagent solution containing α-ketoglutaric acid, L-aspartic acid and NADH is introduced by the pump (9) so that an enzyme reaction caused by GOT in the serum proceeds. The container (2) is passed a plurality of times across an optical path (13), and variation with time of difference between absorbance at 340 nm and 376 nm is measured to determine a reaction rate for the first reaction solution.

Thereafter, the second reagent solution containing L-alanine is introduced by the pump (10) so that an enzyme reaction caused by GPT proceeds, and the reaction rate of this second reaction solution is determined in the same manner. Activity of GOT in the sample is determined from the reaction rate of the first reaction solution, and the sum of activities of GOT and GPT is determined from the reaction rate of the second reaction solution.

FIG. 2



EP 0 041 366 A1

METHOD FOR OPTICALLY ANALYZING A PLURALITY OF ANALYSIS ITEMS

~~BACKGROUND OF THE INVENTION~~

This invention relates to a method for optically analyzing a plurality of items, particularly a method for analyzing a plurality of items by subjecting a sample to enzyme reaction, and then determining the result or progress of the enzyme reaction by a photometer.

In the quantitative analysis of a sample containing many components, particularly that of a metabolic material in body fluid such as blood, analytical methods utilizing an enzyme which acts specifically on a metabolic material have been recently employed. An enzyme reaction proceeds under very mild conditions in a short time. Enzymes have a property of acting merely on a specific material even if it contains many contaminants. Analytical methods utilizing an enzyme reaction having such advantages are employed for biochemical inspection in hospitals, etc.

Conventional photometric methods utilizing an enzyme reaction are generally directed to quantitative analysis of only one analysis item in one sample placed in one reactor vessel, as disclosed, for example, in U.S. Patent No. 3,838,010.

An object of the present invention is to provide a method for optical analysis where a plurality of items can be quantitatively determined for a sample placed in a vessel.

The present invention provides a method where a plurality of enzyme reactions are made to take place sequentially in one vessel; optical characteristics of each reaction solution is measured; the first analysis item is obtained on the basis of the first enzyme reaction; and the second analysis item on the basis of the second enzyme reaction.

One advantage obtainable in embodiments of the present invention is that only a very small amount of a sample may be enough for the analysis of a plurality of items.

It is also possible to provide an efficient analytical method where sampling number can be decreased, so that second or successive sampling operations can be omitted for further analysis items.

Furthermore it is possible for a plurality of enzyme reactions to be utilized for analyzing a plurality of items, and thus the reaction of the first analysis item does not interfere with the reaction of the second analysis item, resulting in very small error in measurement.

According to one preferred embodiment of the present invention, a reagent solution containing an enzyme is added to a sample solution to cause enzyme reaction, and the result of reaction is determined by colorimetric end point method.

According to another preferred embodiment of the present invention, a reagent solution containing a substrate is added to a sample solution to cause enzyme reaction of the substrate with an enzyme contained in the sample solution, and the progress of reaction is determined by rate assay method. Therefore, the concept "concentrations of analysis items" according to the present invention covers the content of components in a sample and the activity of an enzyme in a sample.

According to yet another preferred embodiment of the present invention, the absorbance of a first reaction solution resulting from the addition of a first reagent solution is measured, and then a second reagent solution is added to the first reaction solution to obtain a second reaction solution. The concentration of a first analysis item is obtained on the basis of the absorbance of the first reaction solution. The concentration of the second analysis item is obtained on the basis of the absorbance of the second reaction solution and the absorbance of the first reaction solution. In that case, the volume of the second reaction solution is larger than that of the first reaction solution, and thus, in order to calculate the concentration of the second

analysis item from signals based on both reaction solutions, the absorbance values corrected on an assumption that both reaction solutions have equal volumes are used. That is, either absorbance is to be corrected in accordance with the degree of dilution of the first reaction solution due to the addition of the second reagent solution:

[redacted]

Fig. 1 is a flow diagram schematically showing a structure of one embodiment according to the present invention.

Fig. 2 is a flow diagram schematically showing a structure of another embodiment according to the present invention.

Fig. 3 is a diagram showing a signal-processing system of the embodiment of Fig. 2.

Fig. 4 is a diagram showing the measurement of three analysis items in one sample.

Fig. 5 is a diagram showing a reaction process in the analysis of lactic acid dehydroenzyme (LDH) and leucine aminopeptidase (LAP).

Fig. 6 is a diagram showing a reaction process in the analysis of glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).

[redacted]

Several examples of applying an enzymatic

analytical method to a serum sample will be described below before describing the embodiments according to the present invention.

At first, glucose in serum undergoes the following reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2 \text{ -- (1)}$$

Cholesterol includes an ester form and a free form, and each form undergoes the following reaction. Whole cholesterol is the total of the two forms:

$$\text{Ester form cholesterol} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Free form cholesterol} + \text{Fatty acid} \text{ ---- (2)}$$

$$\text{Free form cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta^4\text{-Cholestenone} + H_2O_2 \text{ ---- (3)}$$

Neutral fat undergoes the following reaction:

$$\text{Neutral fat} + 3H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acid} \text{ ----- (4)}$$

$$\text{Glycerol} + O_2 \xrightarrow{\text{Glycerol oxidase}} \text{Glyceraldehyde} + H_2O_2 \text{ ---- (5)}$$

Phospholipids undergo the following reaction:

$$\text{Phospholipid} \xrightarrow{\text{Phospholipase D}} \text{Choline} \text{ ------------- (6)}$$

$$\text{Choline} + O_2 \xrightarrow{\text{Choline oxidase}} \text{Betaine} + H_2O_2 \text{ -------- } (7)$$

Hydrogen peroxide (H_2O_2) produced in the above-mentioned reactions according to formulae (1), (3), (5) and (7) undergoes reaction according to the following formula (8) by action of peroxidase to produce a red pigment, and thus the reaction can be traced by monitoring by photometer.

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red quinone} \text{ -------- } (8)$$

Example 1

Fig. 1 is a flow diagram schematically showing a structure of the analytical apparatus according to one embodiment of the present invention. When two items of glucose and whole cholesterol are to be analyzed, a first reagent solution containing glucose oxidase, peroxidase, 4-aminoantipyrine, phenol and the like, which are necessary for the above-mentioned reactions of formulae (1) and (8), is added to a predetermined amount of a sample, and after the completion of the reactions of formulae (1) and (8), the absorbance of first reaction solution is measured by colorimetric method, and the concentration of glucose is calculated from the thus obtained absorbance value. Subsequently, a second reagent solution containing enzymes such as

cholesterol esterase, cholesterol oxidase and the like, which are necessary for the reactions of formulae (2) and (3), is added to the above-mentioned first reaction solution. Consequently, the reactions of formulae (2), (3) and (8) take place. For the peroxidase required for the reaction of formula (8), the remaining portion of the first reagent solution is used. After the completion of the reactions of formulae (2), (3) and (8), the absorbance of second reaction solution is measured. The difference between the now obtained absorbance datum and the previously obtained one is proportional to the concentration of whole cholesterol.

Likewise, any of two items can be selected from the glucose, whole cholesterol, free cholesterol, neutral fat and phospholipids, and can be analyzed in one and same reactor vessel by single sampling.

The structure shown in Fig. 1 will be explained below. A flexible chain 51 is loaded with a large number of transparent reactor vessels 52. The chain 51 is comprised of a large number of detachable cylindrical holders rotatably connected to one another. Each of the reactor vessels 52 containing a liquid sample such as a serum sample is charged into each of the holders, and conveyed in a horizontal direction by means of driving sprockets 53 and 54. Both ends of chain 51 may be connected to each other or separated from each other. Chain 51 moves over thermostat bath 50 containing a liquid at a predetermined temperature, while the reactor

vessels are conveyed while their lower parts are immersed in thermostat bath 50. Over thermostat bath 50, there are reagent-adding positions 55 and 56 and photometric positions 61 and 62.

Light beam from a light source 60 is devided in two beams by a mirror system, cast onto the reactor vessels at photometric positions 61 and 62 immersed in the thermostat liquid through light-transmitting windows provided on the side wall of thermostat bath 50, passed through the side wall on the opposite side, and led to multi-wavelength photometer 63 equipped with a concave diffraction grating 65 through one light pass.

Though not shown in the drawing, the light beam having passed through photometric position 61 and the light beam having passed through photometric position 62 are time-shared from each other by a sector or the like, and led to photometer 63 alternately.

A plurality of semiconductor light detectors 67 are arranged at positions corresponding to the respective measuring wavelengths on Rowland's circle 66 of multi-wavelength photometer 63. Electric signal from either light detector is selected by wavelength selector 70, and their differential signal is obtained by means of differential amplifier 71. The differential signal is converted into a digital signal by A-D converter 72, and led through interface 73 to central processing unit 75 for carrying out necessary processings.

First dispenser 80 and second dispenser 82 are

connected to central processing device 75 through interface 74 and interface 73 of controlling mechanism. Analysis items are input into the central processing unit from operating panel 78, and the measured analytical results are displayed on display part 79. Reading-out-memory (ROM) 76 and random access memory (RAM) 77 are provided on central processing unit 75.

First dispenser 80 is provided with discharge pipe 84 extendable over to reagent-adding position 55 and suction pipe 86 insertable into enzyme-containing first reagent solution tank 81. Second dispenser 82 is provided with discharge pipe 85 extendable over to reagent-adding position 56 and suction pipe 87 insertable into enzyme-containing second reagent solution tank 83. In analyzing both items of glucose and whole cholesterol by apparatus of Fig. 1, a first enzyme reagent solution and a second enzyme reagent solution having the following compositions are used.

Composition of first enzyme reagent solution:

Phosphate buffer (pH 7.0)	100m mole/liter
Glucose oxidase	18U/ml
Peroxidase	1.2U/ml
4-Aminoantipyrine	0.8m mole/ml
Phenol	11m mole/liter

Composition of second enzyme reagent solution:

Phosphate buffer (pH 7.7)	5 moles/liter
Cholesterol esterase	2U/liter
Cholesterol oxidase	3U/liter

Methanol	10 moles/liter
Hydroxypolyethoxydodecane	4%

In analyzing glucose and whole cholesterol, the amount of serum sampled into reactor vessel 52 is 5µl; the amount of the first enzyme reagent solution to be added is 500µl; and the amount of the second enzyme reagent solution to be added is 50µl. Absorbance is measured by single beam dual wavelength method. The wavelengths selected by wavelength selector 70 are 505nm and 600nm. Temperature of thermostat bath 50 is maintained at 37°C.

Serum sample is placed in reactor vessel made of transparent material, and then the reactor vessel is loaded onto chain 51.

A vessel for reagent blank and a vessel containing the standard sample of glucose and that of whole cholesterol are placed at the head of a series of reactor vessels for sample. Before the measurement of analysis sample, working curves for both analysis items are obtained from the measured values of the reagent blank and the standard samples.

When chain 51 moves and reactor vessel 52 containing the serum sample reaches first reagent-adding position 55, first dispenser 80 is operated and first enzyme reagent solution is charged into the reactor vessel from discharge pipe 84.

The sample thus mixed with the reagent solution immediately undergoes reaction according to formulae (1) and (8). When reactor vessel 52 is

intermittently conveyed to photometric point 61, light is cast onto the reactor vessel from light source 60, and the transmitted light is dispersed into spectra by concave diffraction grating 65 of multi-wavelength photometer 63, and the intensity of specific wavelength light is measured. The signal of light intensity serves to calculate the corresponding glucose concentration on the basis of the working curve obtained in advance and the glucose concentration is diplayed on display part 79. When the same reactor vessel advances by one more step and reaches second reagent-adding position 56, second dispenser 82 is put into operation and the second enzyme reagent solution is charged into the reactor vessel from discharge pipe 85, and then the sample solution thus admixed immediately undergoes reactions according to formulae (2), (3) and (8). When the reactor vessel is intermittently conveyed to photometric position 62, light is cast onto the reactor vessel from light source 60, and the transmitted light is dispersed into spectra by multi-wavelength photometer, and a signal based on the light intensity of same specific wavelength light as above is obtained. The signal value based on the light intensity measured for the same sample at photometric position 61 prior to the addition of the second enzyme reagent solution has been memorized by RAM, and therefore a difference between the memorized signal value and the signal value now obtained due to the reaction caused by the addition of the second enzyme

reagent is proportional to the concentration of whole cholesterol. Accordingly, the concentration of whole cholesterol in the analysis sample can be calculated from both signal values and the working curve of whole cholesterol obtained in advance and then displayed.

In calculating the cholesterol concentration, correction is made for comparison of the signal from photometric position 61 with the signal from photometric position 62 under the same conditions in the signal processing part including the central processing unit. That is, the volume of sample solution before the addition of the second enzyme reagent is different from that after the addition, and thus at least either signal must be corrected to a value obtainable when the volumes are supposed to be equal to each other, and thereafter the cholesterol concentration must be calculated.

In the present Example, the light signal from first photometric position 61 and the light signal from second photometric position 62 are to be measured only for equal wavelength light, but measurement can be carried out for different wavelength lights. In the case of different wavelength lights, measurement is carried out for one specific wavelength for a first analysis item and for another wavelength light for a second analysis item, where both one specific wavelength light and another wavelength light are taken up from the light from first photometric position 61, while another

wavelength light is taken up from the light from second photometric position 62. When the present invention is applied to a rate assay method, correction should be made for a change with time in addition to the correction for the change in the volume of solution.

According to the above-mentioned embodiment, analysis of two items corresponds to a single sampling, and two items can be analyzed in one reaction line.

Example 2

Another embodiment according to the present invention will be described below, referring to Fig. 2.

Reaction disc 1 has, on the circumferential edge, a plurality of, for example, 40 light-transmitting reactor vessels 2 serving also as measuring cells, and can be rotated clockwise either by one full turn or by divisional pitch-by-pitch turn around rotary shaft 3.

Sample table 4 has a plurality of sample containers 5 on its circumferential edge, and can be intermittently rotated clockwise step by step around rotary shaft 6. Pipetting of a sample is carried out by pipette 7 provided with sampling probe 8, and the first and second enzyme reagents are poured into the vessels portion by portion by metering pumps 9 and 10. Photometer 11 is of the same multi-wavelength photometer type having a plurality of detectors as that of photometer 63 shown in Fig. 1, and arranged to face light source lamp 12 through a line of the reactor vessels so that light

beam 13 from the light source can pass through the lines of reactor vessels 2, while the reaction disc is in rotation.

When the reaction disc 1 is at rest, arrangement is made so that light beam 13 of the photometer can pass through the center of a reactor vessel, for example, at the 31st position as counted clockwise from the sample-discharge position 25, to reactor vessel. A plurality of solution-discharge pipes 26 and a plurality of washing water-discharge pipes 27 are provided between the position of light beam 13 and sample-discharge position 25 so that the pipes can be inserted into or removed, from the reactor vessels. The pipes are also connected to solution-discharging device 28 and washing device 29, respectively.

The whole structure of electric-signal-processing system 40 is comprised, as shown in Fig. 3, of multiplexer 14, logarithm conversion amplifier 15, A/D converter 16, central processing unit 17, reading-out memory 18, read-out and write memory 19, printer 20, operating panel 21 and mechanism-driving circuit. They are connected to bus line 23.

Now, description will be made of operations according to the present embodiment. When sample container 5 containing a sample to be analyzed, such as serum, arrives at sampling position 30, the tip end of probe (suction-and-discharge pipe) 8 of pipette 7 is inserted into sample container 5, and a predetermined

amount of serum is taken up by suction and retained inside probe 8. Thereafter, probe 8 moves to discharging position 25 on reaction table 1, and then charges the serum retained therein into reactor vessel 2 at sample-receiving position 25. When the sampling operation is completed, reaction disc 1 is actuated to rotate clockwise continuously or intermittently only by such necessary angle of turn that total number plus one of the reactor vessels 2 on reaction disc 1 can pass through discharge position 25, that is, by 369°.

Owing to the rotation of reaction disc 1, reactor vessel 2 containing the sample taken up by sampling operation rests at the position only by one pitch, that is, only by 9°, far from discharge position 25, that is, first reagent-adding position 31. During the rotation of reaction disc 1, all of reactor vessels 2 on reaction disc 1 pass across light beam 13. When each of reactor vessels 2 passes through light beam 13, light absorption measurement of each sample solution is carried out by spectroscope 11. From the output of spectroscope 11, signals with wavelength now necessary for the measurement are selected by multiplexer 14, and then put into central processing unit 17 through A/D converter 16, and memorized in reading-and-writing memory 19.

Suppose that the period for rotation and rest of reaction disc 1 be, for example, 30 seconds. Operation and rest for the 30 seconds is repeated as one

cycle. With repetitions of the cycle, a specific sample taken up can take a clockwise one-pitch advanced position when reaction disc 1 is at rest.

Metering pump 9 is directed to introducing the first enzyme reagent solution in tank 35 into reactor vessels, and metering pump 10 is directed to introducing the second enzyme reagent solution in tank 36 into reactor vessels. The first and second enzyme reagent solutions have the same compositions as used in the embodiment of Fig. 1. The discharge pipes 33 and 34 of metering pumps 9 and 10, respectively, are vertically movable, and a little descend when the reagent solutions are discharged. Discharge pipe 33 of metering pump 9 and discharge pipe 33 of metering pump 10 are provided over reactor vessel 2 at reagent-adding position 31, that is, the 1st position counted clockwise from discharge position 25, and over reactor vessel 2 at reagent-adding position 32, that is, the 16th position counted clockwise from discharge position 25, respectively, for example, when reaction disc 1 is at rest. That is, a given sample in reactor vessel 2 is admixed with the first enzyme reagent at reagent-adding position 31, whereby enzyme reaction of first group is initiated, and when the relevant reactor vessel reaches reagent-adding position 32 at the 15th cycle, the second enzyme reagent is added to the reactor vessel by metering pump 10, whereby the second enzyme reaction is initiated. When reactor vessel 2 moves its position at the rest of

reaction disc 1 across light beam 13 to between light beam 13 and sample-receiving position 25 with further repetitions of the cycle, measurement of the given sample in the reactor vessel can be regarded as completed, and the given sample solution is discharged by suction through discharge pipe 26 by discharging device 28. Subsequently, washing water (usually distilled water) is charged into the reactor vessel through wash water discharge pipe 27 from washing device 29. At the subsequent rest of reaction disc 1, the washing water is discharged from the reactor vessel in the same manner as above ultimately, and the washed reactor vessel is reused for another sample at sampe-receiving position 25 with further repetitions of the cycle. The foregoing operations are carried out by controlling the respective mechanism parts by central processing unit 17 through mechanism part-diving circuit 22 according to the program of read-out memory 18. Operating panel 21 is used for such operations as input of measuring conditions, start and discontinuation of measurement, etc.

Suppose that reaction disc 1 have a rest time of 9.5 seconds and a rotation time of 20.5 seconds in one cycle of the foregoing operation. Reaction progress of the given sample can be measured 31 times at intervals of 29.5 seconds, and thus data resulting from the measurements for 15 minutes 15 seconds are memorized in read-out and write memory 19. Central processing unit 17 operates according to the program of read-out memory

18, extracts the necessary data from 31 measurement data in read-out and write memory 19 according to the predetermined program, and gives output to printer 20 after processing such as concentration calculation, etc.

Description will be made a little in detail below, referring to an example, where the apparatus according to the embodiment of Fig. 2 is applied to analysis of two items of glucose and whole cholesterol.

On the basis of 31 absorbance data for each sample which have been memorized in read-out and write memory 19, concentration is calculated in the following manner according to predetermined program. That is, suppose that 16th absorbance datum be E_{16} and 31th datum E_{31}.

Glucose concentration Y_1 will be expressed as follows:

$$Y_1 = \frac{C_S}{E_{16}^S - E_{16}^O} (E_{16} - E_{16}^O)$$

Whole cholesterol concentration Y_2 will be expressed as follows:

$$Y_2 = \frac{C_S'}{E_{31}^S - KE_{16}^S} (E_{31} - KE_{16})$$

wherein C_S and C_S' are glucose concentration and cholesterol concentration, respectively, of standard

solution used for preparing a working curve, and memorized as input from operating panel 21; E^0_{16} is 16th data for the reagent blank; E^S_{16} and E^S_{31} are 16th data for glucose and 31th data for whole cholesterol of standard solution; K is a correction factor for the amount of solution and in this case, K = 505/555 because the amount sample is 5μl, that of first enzyme reagent solution 500μl and that of second enzyme reagent solution 50μl.

The present invention is applicable not only to the analysis of two components but also to that of three or more components. For example, in order to amalyze three components by application of the apparatus of the embodiment shown in Fig. 2, a third enzyme reagent-adding position is provided between second reagent-adding position 32 and light beam 13. For example, in analyzing three components of glucose, whole cholesterol and neutral fat, after the above-mentioned analysis of two components of glucose and the whole cholesterol, lipoprotein lipase and glycerol oxidase are added as third reagents to the reaction solution to complete the reactions of formulae (4), (5) and (8), and the concentration of neutral fat is calculated from the difference between the absorbances before and after the addition of the third reagents.

When the absorbance of the reaction solution in this case is traced with time, the results will be as given in Fig. 4. The magnitude of a, b and c in Fig. 4

are proportional to the respective concentrations of glucose, whole cholesterol and neutral fat.

I, II and III in Fig. 4 show the points of time of adding the first, second and third enzyme reagents, respectively.

Although in embodiments shown in Fig. 1 and Fig. 2, the same measuring wavelength is used for analyzing two components, different measuring wavelengths can be selected for analyzing the first component and for analyzing the second component.

In this case, data with two different wavelengths can be obtained as 16th absorbance datum, or 15th absorbance datum E_{15} may be obtained as data for the first component with a wavelength different from the measuring wavelengths for E_{16} and E_{31}.

In this case, concentration Y_1 of the first component can be calculated as follows:

$$Y_1 = \frac{C_S}{E_{15}^S - E_{15}^0} (E_{15} - E_{15}^0)$$

Concentration Y_2 of the second component can be expressed by the following equation:

$$Y_2 = \frac{C_S'}{E_{31}^S - {}_K E_{16}^S} (E_{31} - {}_K E_{16})$$

A few embodiments of measuring the activity of

enzyme contained in a sample by rate assay method will be described below. The analytical apparatus shown in Fig. 2 will be used in the following embodiments.

Example 3

In the present embodiment, a method for analyzing two analysis items of lactate dehydrogenase (LDH) and leucine aminopeptidase (LAP) contained in a serum sample is applied to the apparatus in Fig. 2. As examples of suitable reagent compositions in this case, solutions having the following compositions are used, where NADH means reduced form nicotineamide adenine dinucleotide.

Composition of first reagent solution:

Pyruvic acid	0.6m moles/liter
Phosphate buffer (pH 7.5)	50m moles/liter
NADH	0.18m moles/liter

Composition of second reagent solution:

L-leucine-p-nitroanilide	3.2m moles/liter
Phosphate buffer (pH 7.5)	400m moles/liter

Measuring conditions for the apparatus are as follows:

Amount of sample	20μl
Amount of first reagent	500μl
Amount of second reagent	250μl
Reaction temperature	25°C
Measuring wavelengths 1	340nm/376nm
Measuring wavelengths 2	405nm/505nm

When the above-mentioned first and second reagents are placed in solution tank 35 for metering pump 9 and solution tank 36 for metering pump 10, respectively, and sample table 4 is loaded with the sample, then an instruction "start analysis" is given from operating panel to actuate the apparatus.

Reaction in reactor vessel 2 is traced. Reaction proceeds according to the following formula (9) from the point of time of mixing the sample with the first reagent solution.

$$\text{Pyruvic acid} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactic acid} + \text{NAD} \text{ -------- } (9)$$

The second reagent is subsequently added thereto after 7.5 minutes, and reaction starts according to the following formula (10) in parallel with the reaction according to the above formula (9).

$$\text{L-leucine-p-nitroanilide} + H_2O \xrightarrow{\text{LAP}} \text{L-leucine} + \text{p-nitroaniline} \text{ ------ } (10)$$

The rate of reaction of formula (9) can be determined by tracing the absorbance of NADH according to the single beam dual-method at 340nm/376nm, and is proportional to the activity of LDH. The rate of reaction of formula (10) can be determined by tracing formation rate of p-nitroaniline through the absorbance according to the single beam dual-wavelength method, and

is proportional to the activity of LAP.

In the case of the combined use of these two pairs of wavelengths, as shown in Fig. 5, the measuring wavelengths are changed from 340nm/376nm for the measurement of the reaction by the first reagent to 405nm/505nm at the point of time of adding the second reagent. After the change, the components for the reaction of formula (9) contain no such components that substantially absorb the relevant wavelengths, and thus only the reaction of formula (9) can be traced. In Fig. 5, A is the point of time of adding the first reagent. Between the point of time A and the point of time B, LDH reaction takes place, and after the point of time B, LAP reaction takes place.

Example 4

Description will be made below of another embodiment of a method for analyzing two analysis items of glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase. As examples of suitable reagent compositions in this case, solutions of the following compositions can be used.

Composition of first reagent solution:

α-Ketoglutaric acid	18m moles/liter
L-aspartic acid	200m moles/liter
NADH	0.18m moles/liter
MDH	≧0.6U/ml
LDH	≧1.2U/ml

Phosphate buffer (pH 7.4)	80m moles/liter

Composition of second reagent solution:

L-alanine	6.4m moles/liter
Phosphate buffer (pH 7.4)	80m moles/liter

The measurement conditions for the apparatus are as follows:

Amount of sample	20μl
Amount of first reagent	350μl
Amount of second reagent	50μl
Reaction temperature	25°C
Measurement wavelengths	340nm/376nm

When said method is applied to the apparatus in Fig. 2, the reactions of formulae (11) and (12) proceed after the addition of the first reagent.

$$\text{L-aspartic acid} + \alpha\text{-Ketoglutaric acid} \xrightarrow{\text{GOT}} \text{Glutamic acid} + \text{Oxalacetic acid} \text{ ----- } (11)$$

$$\text{Oxalacetic acid} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malic acid} + \text{MAD} \text{ -- } (12)$$

When the second reagent is subsequently added, the reactions of formulae (13) and (9) proceed in parallel with the reactions of formulae (11) and (12).

$$\text{L-alanine} + \alpha\text{-Ketoglutaric acid} \xrightarrow{\text{GPT}} \text{Glutamic acid} + \text{Pyruvic acid} \text{ ----- } (13)$$

The rate of the reaction of formula (11) is

proportional to GOT concentration in the sample and a decreasing rate of NADH in the reaction of formula (12) coupled with the reaction of formula (11), and a decreasing rate of NADH can be determined from the absorbances at 340nm/376nm. That is to say, as shown in Fig. 6, an absorbance change per minute, X_1, can be obtained from 15 absorbance data in 15 cycles for 7.5 minutes after the addition of the first reagent, and the activity Y_1 of GOT can be obtained as the following formula:

$$Y_1 = \frac{X_1 \times V_1 \times 1{,}000}{\varepsilon \times d \times v} \quad \text{------------------} \quad (14)$$

wherein V_1 is a total volume of reaction solution (V = 370μl); ε is a molecular absorption coefficient (ε = 4.20); d is length of the optical path (d = 1cm); and v is a volume of samples (v = 20μl).

Thus, Y_1 in formula (15) will be as follows:

$$Y_1 = X_1 \times \frac{370 \times 1{,}000}{4.20 \times 1 \times 20}$$
$$= X_1 \times 4{,}405 \quad \text{--------------} \quad (15)$$

An absorbance change per minute, X_2, can be obtained from 15 absorbance data after the addition of the second reagent and is proportional to the sum (Y_2) of the activities of GOT and GPT. That is to say,

$$Y_2 = \frac{X_2 \times V_2 \times 1{,}000}{\varepsilon \times d \times v} \quad \text{---------------} \quad (16)$$

and since $V_2 = 420\mu l$,

$$Y_2 = X_2 \times \frac{420 \times 1,000}{4.20 \times 1 \times 20}$$

$$= X_2 \times 5,000 \qquad (17)$$

Accordingly, the activity Y_3 of GPT can be obtained from the following equation:

$$Y_3 = Y_2 - Y_1$$

In Fig. 6, A is the point of time for adding the first reagent, and B is the point of time for adding the second reagent.

CLAIMS

1. A method for optically analyzing a plurality of items in a sample solution, which comprises the steps of

(i) preparing a first reaction solution by mixing the sample solution with a first reagent solution capable of causing a first enzyme reaction;

(ii) obtaining a first measurement corresponding to the optical characteristics of the said first reaction solution;

(iii) preparing a second reaction solution by adding a second reagent solution capable of causing a second enzyme reaction to the said first reaction solution;

(iv) obtaining a second measurement corresponding to the optical characteristics of the said second reaction solution;

(v) determining the concentration or other characteristic of a first analysis item in dependence on the optical characteristics of the said first reaction solution; and

(vi) determining the concentration or other characteristic of a second analysis item in dependence on the optical characteristics of the said second reaction solution.

2. A method according to claim 1 wherein the value of at least one of said first and second measurements is corrected in dependence on the degree of dilution of the first reaction solution effected by the addition of the said second reagent solution.

3. A method according to claim 1 or claim 2 wherein a concentration of the said second analysis item is determined on the basis of the difference between the said second measurement and the said first measurement.

4. A method according to any one of claims 1 to 3 wherein said first and second reagent solutions each contain an enzyme, and said first and second measurements are obtained by obtaining a signal corresponding to the light absorption of the said first and second reaction solutions respectively, the concentration of the first analysis item being determined on the basis of the said signal relating to the first reaction solution, and the concentration of the second analysis item being determined on the basis of the said signal relating to the second reaction solution and the signal relating to the first reaction solution.

5. A method according to claim 4 wherein an absorption wavelength used for the second reaction solution is identical to one used for the first reaction solution.

6. A method according to claim 4 wherein the first reaction solution is subjected to measurement of light absorption at each of two wavelengths, and the second reaction solution is subjected to measurement of light absorption at a wavelength identical to one of the said two wavelengths.

7. A method according to any one of claims 1 to 3 wherein said first and second reagent solutions react with respectively a first and a second enzyme contained in the sample solution, and said first and second measurements are obtained by optically measuring the reaction rate of respectively the first and second reaction solutions, and wherein the activity of the said first enzyme is determined on the basis of the reaction rate of the said first reaction solution, and the activity of the said second enzyme is determined on the basis of the reaction rate of the said second reaction solution.

8. A method according to claim 7 wherein the activity of the second enzyme is determined on the basis of the difference between the reaction rate of the second reaction solution and that of the first reaction solution.

9. A method according to claim 7 wherein the reaction rates of both the first reaction solution and the second reaction solution are determined on the basis of a change with time in values measured by single beam dual-wavelength method.

10. A method according to any one of claims 1 to 3 which includes the steps of

conveying a transparent container containing the sample to a position where said first reagent solution is added so as to prepare said first reaction solution,

conveying said container containing the first reaction solution so that the container passes across an optical path of a photometer thereby obtaining a signal corresponding to optical characteristics of the first reaction solution by means of said photometer,

conveying said container containing the first reaction solution to a position where the said second reagent solution is added to prepare a second reaction solution,

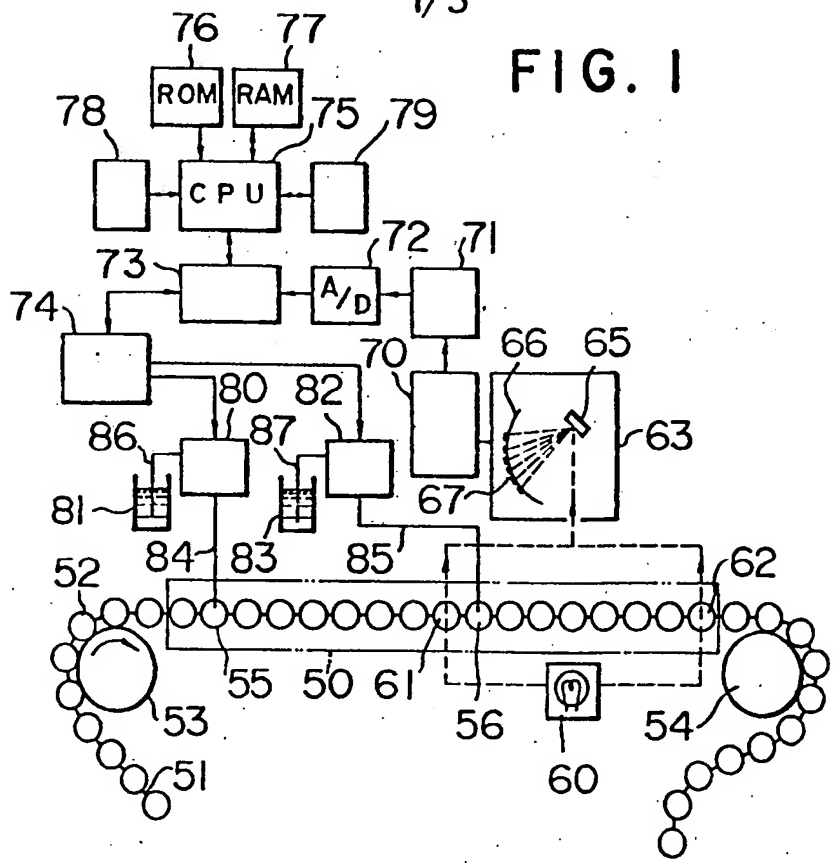
conveying said container containing the second reaction solution so that the container passes across an optical path of said photometer thereby obtaining a signal corresponding to optical characteristics of the second reaction solution by means of said photometer,

determining the concentration of the first analysis

item from the signal thus obtained for the first reaction solution, and

determining the concentration of the second analysis item from the signal thus derived for the second reaction solution.

FIG. 1



76
77
ROM
RAM
78
75
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CPU
72
71
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A/D
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80
82
86
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81
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85
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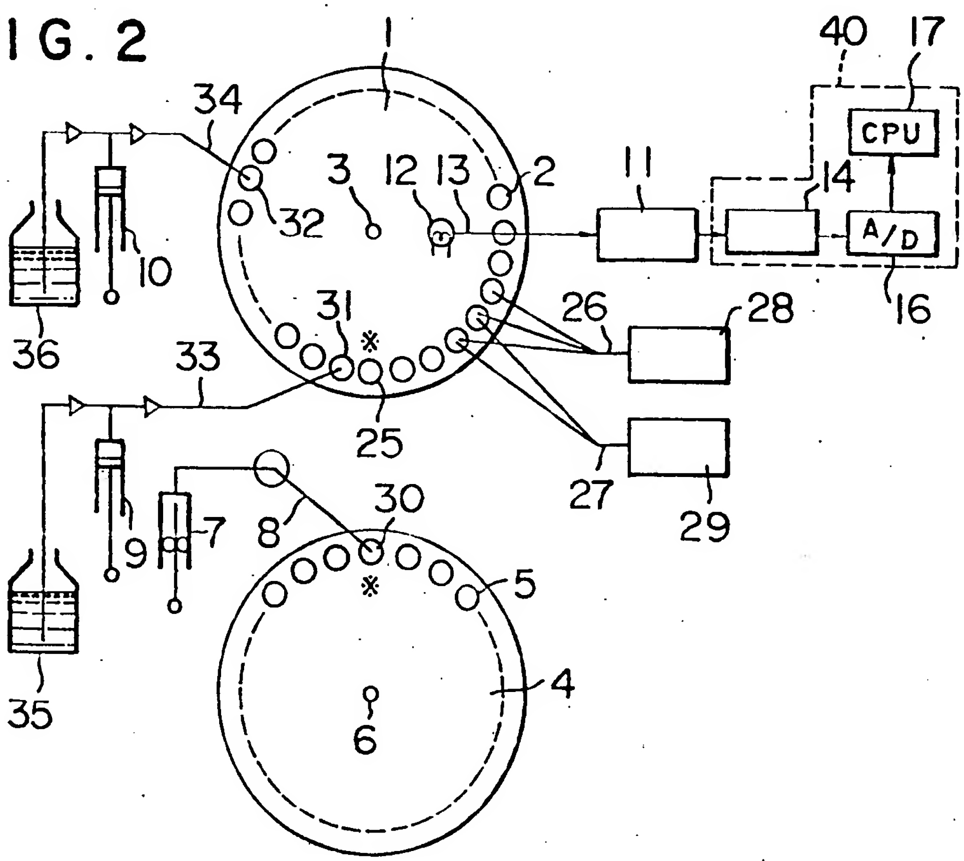
60
51

FIG. 2



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CPU
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13
2
11
14
32
A/D
10
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16
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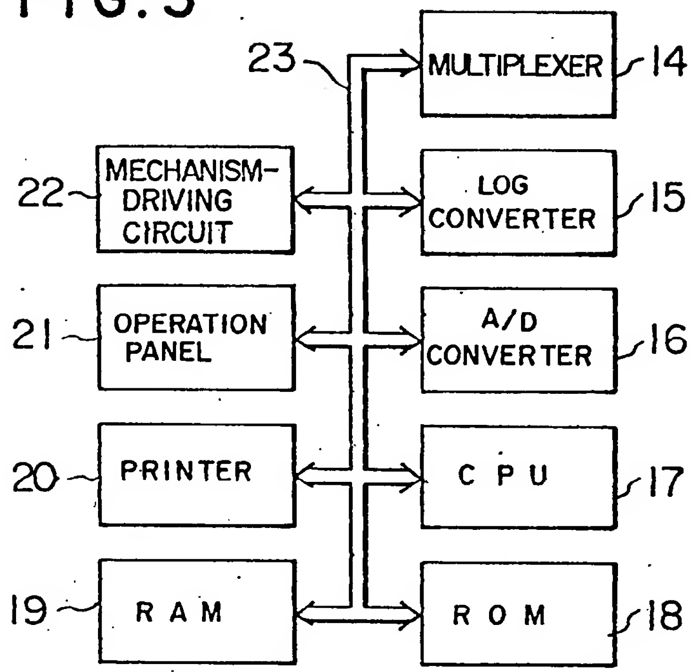
6

FIG. 3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MULTIPLEXER
14
22
MECHANISM-DRIVING CIRCUIT
LOG CONVERTER
15
21
OPERATION PANEL
A/D CONVERTER
16
20
PRINTER
C P U
17
19
R A M
R O M
18

FIG. 4

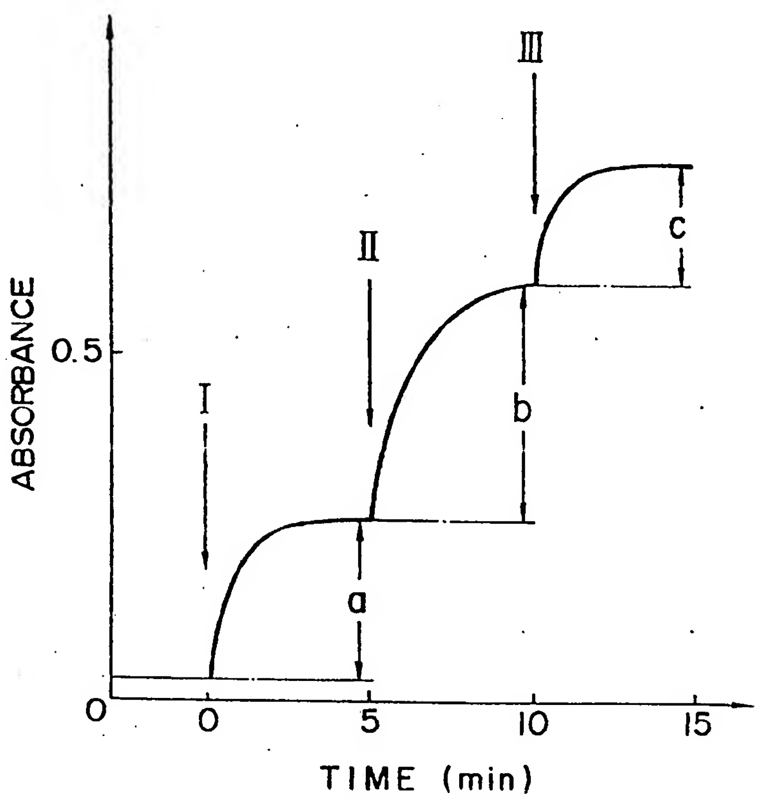

ABSORBANCE
0.5
0
I
II
III
a
b
c
0
5
10
15
TIME (min)

FIG. 5

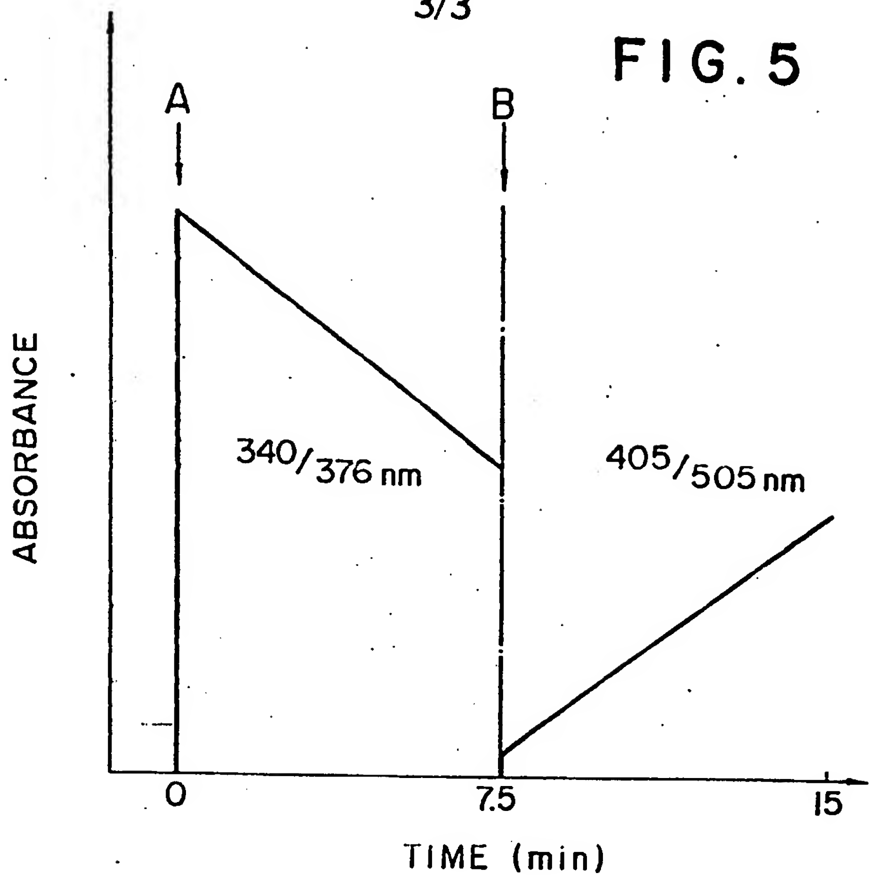
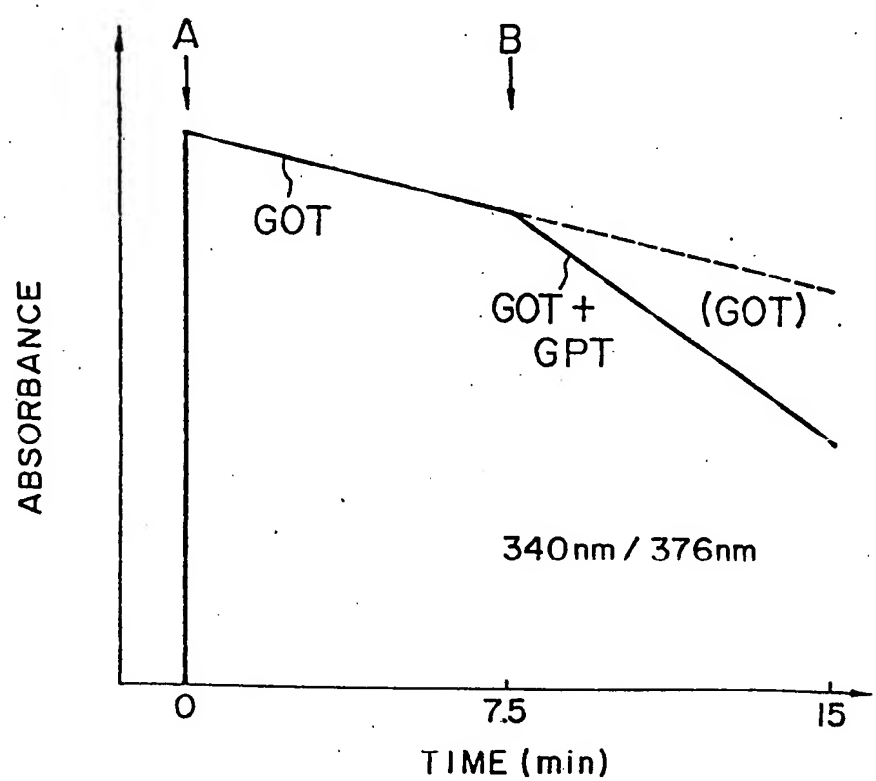

A
B
ABSORBANCE
340/376 nm
405/505 nm
0
7.5
15
TIME (min)

FIG. 6


A
B
GOT
GOT +
GPT
(GOT)
ABSORBANCE
340nm / 376nm
0
7.5
15
TIME (min)

0041366

European Patent Office

EUROPEAN SEARCH REPORT

Application number
EP 81 30 2354

Category	DOCUMENTS CONSIDERED TO BE RELEVANT — Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.³)
	NL - A - 77 09 175 (VITATRON SCIENTIFIC) * example 1 * & GB - A - 2 043 244	1	G 01 N 35/02 33/52 21/27
D	US - A - 3 838 010 (F.E. HAMMER) * column 11, lines 9-21 *	1	
	US - A - 4 063 816 (N. JTOI et al.) * column 6, lines 16-39; figure 1 *	6,7,10	TECHNICAL FIELDS SEARCHED (Int. Cl.³)
	US - A - 3 621 215 (H. NETHELER et al.) * column 5, lines 21-55 *	7	G 01 N 35/00 35/02 21/27 21/31 21/59 21/75 33/52
	US - A - 4 155 978 (T. NAONO et al.) * column 2, line 64 to column 3, line 16; figure 1 *	10	
	FR - A - 2 097 329 (CIE. GENERALE D'AUTOMATISME) * page 2, lines 5-35; figure *	6,9	CATEGORY OF CITED DOCUMENTS X: particularly relevant A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: conflicting application D: document cited in the application L: citation for other reasons
A	CLINICAL CHEMISTRY, vol. 18, no. 12, 1972 ./.	1,7	&: member of the same patent family, corresponding document

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	09-09-1981	KEMPIN

EPO Form 1503.1 06.78

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European Patent Office

EUROPEAN SEARCH REPORT

Application number
EP 81 30 2354

-2-

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.3)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	D.W. MOSS: "The Relative Merits and Applicability of Kinetic and Fixed-Incubation Methods of Enzyme Assay in Clinical Enzymology" pages 1449-1454 * conclusions * -------		
			TECHNICAL FIELDS SEARCHED (Int. Cl.3)